An Overview of the Capillary Electrophoresis Process at the DOE Joint Genome Institute Production Genomics Facility: The Dual Operation of the AB 3730xl & GE MegaBACE 4500 DNA Sequence Analyzers

Christopher Daum, Lena Philip, Danielle Mihalkanin, Cailyn Spurrell, Don Miller, Susan M. Lucas, Alex Copeland, Damon Tighe, Eric Abbott, Marlon Arcaina, Nicholas Eattock, Melanie Lafrades, Albert Linkowski, Adrienne Loero-Pequignot, Andy Yuen, Mathew Zane

DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

ABSTRACT

At the center of the Department of Energy's (DOE) Joint Genome Institute (JGI) Production Genomics Facility (PGF), lies a highly efficient and automated production line devoted to the generation of high-quality genomic DNA sequence. The JGI utilizes a dual platform of DNA sequence analyzers within its Production Capillaries group: seventy Applied Biosystems 3730xl and thirty-six GE Healthcare MegaBACE 4500 instruments. The Capillaries group is comprised of eleven employees that are responsible for operating and maintaining both platforms; the group is also involved in the daily monitoring of performance stats and the troubleshooting of DNA sample and instrument related issues. The operation of these high-throughput fluorescence-based DNA sequence analyzers will be assessed on the strengths and benefits of each platform, including instrument overviews of operational parameters and mechanical/component specifications. In addition, instrument setups for production operation, operation schedules, loading, and maintenance strategies as well as the various sequencing strategies for each platform will be compared. Throughput numbers and sequencing quality results will be presented.

Introduction

The MegaBACE DNA Analysis platform has been an indispensable component of the JGI production sequencing line for several years; with the inception of this capillary based platform in 1999, significant advances were made over the industry standard slab gel instruments of the time – in areas of automation, workflow, and operation. The current MB4500 platform offers much improved sample throughput with the ability to process 384 sample lanes in a single run and improved sequence quality over its predecessors with updated components.

In 2002 the ABI3730xl was released - this highly automated DNA analyzer was poised to shift the paradigm of high-throughput sequencing as the JGI and genomics facilities world-wide adopted this new platform; a new industry standard had been set.

The JGI has chosen to utilize both the MB4500 - 36 instruments, and the ABI3730xl - 70 instruments. The highly automated ABI3730xl allows for 24hr-7day/week processing of sample plates, with only limited interaction by a small staff of Technicians who load/unload samples and change out reagents a few times per week. Alternatively, the 384-capillary array system of the MB4500 allows for high sample throughput on each sequencing run performed, however it requires manual interaction with a Technician to load each run.

The MB4500 uses an efficient high power solid-state laser, which mated to the scanning confocal optics system provides enhanced detection sensitivity for long reads; attaining on average 100bases more per lane (based upon JGI run parameters). Additionally, the MB capillary arrays and solid-state laser achieve much longer operational lifetimes when compared to the arrays and argon laser of the ABI. Although in JGI production, the MB instruments have an operational uptime efficiency of 97.9% compared to 98.8% for the ABIs; downtime on both platforms is primarily due to instrument errors.

Capillary Operators *Left to Right*: Andy Yuen, Nicholas Eattock, Donald Miller, Matthew Zane, Adrienne Loero-Pequignot, Christopher Daum, Marlon Arcaina, Melanie Lafrades, Lena Philip, Albert Linkowski. *Not Pictured*: Eric Abbott, Cailyn Spurrell, Dennis Schuster, Amber Nivens

Group History

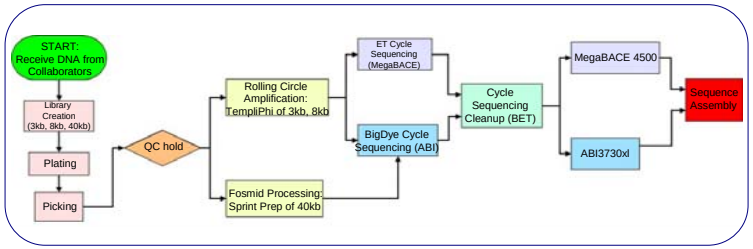
The Production Capillaries group was established in 1999 with the operation of 84 MegaBACE 1000 instruments. The group acquired its first 5 ABI3730xl instruments in May 2002, and by 2004 70 instruments total had been brought online. The MegaBACE 1000s were replaced with 21 MegaBACE 4000s in early 2002 and an additional 15 were brought online in 2004. In 2003 two MegaBACE 4000s were upgraded to the developmental MegaBACE 4500 platform. After extensive testing and development, all of the MegaBACE 4000s were upgraded to MegaBACE 4500s; a process that was completed in early 2005.

The capillaries group was absorbed into the QC group in December 2002 and capillary operators functioned as both instrument operators and QC members. Operators not only ran the instruments, but ran electrophoresis gels, engaged in the troubleshooting of statistics and production line problems, and ran experiments. In January 2006 after some facility-wide restructuring, the capillaries group once again became an autonomous group within the production line under the supervision of Chris Daum. While the capillaries group is autonomous, members still partially function as a QC group and continue to run gels and troubleshoot. Operators also continue to run experiments, but they are tailored more towards capillary improvement as opposed to general QC experiments.

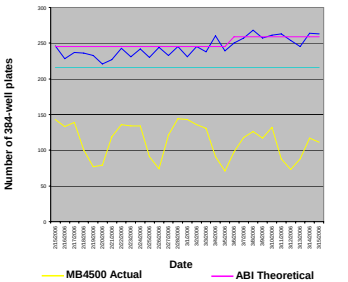
AB 3730xl DNA Analyzer

MegaBACE 4500

JGI Production Process Summary

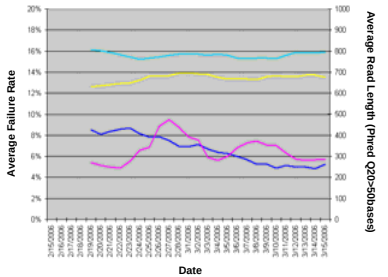


JGI Throughput Comparison of 36 MB4500s vs. 70 ABI3730s



Maximum Platform Throughput/Day: The maximum daily ABI throughput is 265 plates (3.789 plates / instrument / day). The maximum daily MegaBACE capacity varies during the week. Monday maximum capacity is 118 plates / day. Maximum capacity Tuesday – Friday is 144 plates/day. Maximum capacity Saturday is 115 plates/day and the maximum capacity on Sunday is 79 plates / day. The MegaBACE theoretical throughput can not be reached because the MegaBACE platform currently doesn't run on third shift. Actual & theoretical throughput based upon current JGI run parameters, with 36 MB4500 & 70 ABI3730xl instruments in operation.

Performance Comparison of ABI 3730xl & MegaBACE 4500 Platforms



Conclusion

The JGI has chosen to utilize both the MegaBACE 4500 and the ABI3730xl DNA sequencer analyzer instruments, and has found the running of these competing platforms to be beneficial to the production sequencing process. The highly automated ABI3730xl allows for 24hr-7day/week processing of sample plates, with only limited interaction. Alternatively, the 384-capillary array system of the MB4500 allows for high sample throughput during each sequencing run performed by a Technician, and its solid-state laser mated with the scanning confocal optics system provides enhanced detection sensitivity for longer reads. The MB platform also offers slightly reduced per lane cost when comparing the associated operational and sample preparation costs for each platform at the JGI; this reduced operational cost is primarily due to the long lifetimes achieved by the MB capillary arrays. Moreover, these two DNA sequencing platforms complement each other in the sequence they produce - thereby providing for better coverage when sequence derived from both platforms are used in genomic project assemblies at the JGI.

Instrument Error Response

Capillary operators are alerted of instrument errors in a variety of ways. When a MegaBACE errors, it usually occurs while the operator is attempting to follow the command prompts while loading the machine. The MegaBACE software initializes a pop-up that notifies the user that an error has occurred, where the operator then has the option to respond to the error and correct it. When the error can not be corrected the service engineer is notified.

ABI 3730xl errors are found in a different way. Because the ABIs operate autonomously, errors usually occur when the operator is not close to the instrument. Therefore each instrument is connected to a system monitoring program called BioMonitor and when an error occurs notifications are sent out via email and to pagers that the operators carry. When BioMonitor is not working correctly, and notifications are not sent, then down instruments are located during routine walk-throughs. Operators are trained to fix many minor errors and some major errors themselves; however, repeating errors or serious system errors and failures (i.e.: laser, camera, electronics) are reported to the service engineer.

Service Engineers

Donald Miller: Donald Miller is the service engineer for the MegaBACE 4500s. He has been a JGI employee since Jan. 2002, and has completed training offered through Amersham (GE Healthcare) in order to be certified to work on the MegaBACE platform. He performs all of the scheduled preventative maintenance and troubleshoots all instrument errors that occur. When errors occur during hours that Don is not available, the instruments are stored until he can look at them.

Dennis Schuster: Dennis Schuster is the service engineer for the ABI3730xls. He has been an Applied Biosystems employee for 11 years, and has worked on-site at JGI 5 days / week since Sept. 2005. He performs all of the scheduled ABI preventative maintenance as well as troubleshoots 99% of the instrument errors that occur. ABI operators fix minor errors during hours when Dennis is not on-site.

JGI Quick Reference – Operational Parameters & Specifications

Operational Parameter	ABI 3730xl Specification	MegaBACE 4500 Specification
Run Parameters	8.5kV for 75min	9.9kV for 215min
Injection Parameters	1.5kV for 15sec	2.0kV for 36sec
Operating Temperature	60°C	55°C
Sieving Matrix	POP-7 polymer	VI LPA
Capillary Array Type	- 50 cm - 96 capillaries - 150 micron o.d. & 50 micron i.d.	- 76 cm - 384 capillaries - 200 micron o.d. & 75 micron i.d.
Sample Line Throughput (based upon JGI Run parameters)	1440 samples/day	2304 samples/day
Laser power type	25mW argon-ion	100mW solid-state
Laser accessories (cooling)	Forced HVAC system with negative pressure hookup to draw air from system	None - no external Power Supply Fan module or HVAC system required
Optical system – Excitation & Detection	Its capillary dual-side laser excitation & CCD camera detection	Scanning confocal optics for its capillary laser excitation & detection by PMTs
Instrument accessories	- UPS for backup during power outages - PC - Monitor - Barcode Scanner	- UPS for backup during power outages - PC - Monitor - Barcode Scanner - High & Low pressure N_2 systems
Reagent Materials Handling	- Automated sieving matrix delivery pump & on-board reagents for 2 day unattended operations. - Integrated auto-sampler & sample-plate stacker (10 plate capacity)	- Technician loaded sieving matrix & reagents for each run performed. - Individually loaded sample plates by Technician
Sequencing Chemistry	BigDye v3	D Fluorescent ET dye terminators
Instrument Software	Unified Data Collection v3.0	Instrument Control Manager v4.2
Basecalling Software	Sequencing Analysis v5.2 with 3"MPOP"LR basecaller	Sequencer Analysis v4.0 with Cimarron 3.12 basecaller

Weekly ABI Tasks

ABI Tasks	M	T	W	R	F
Change tanks	X		X		X
Load Plates	X	X	X	X	X
Maintenance		X		X	
Array QC	X	X	X	X	X
Array Changes	As needed				

The weekly tasks are divided amongst the entire capillaries group. Other minor tasks include reagent preparation, resolving data transfer issues and troubleshooting minor instrument errors.

MB4500 Run Schedule

Sample MegaBACE run schedule: Four runs / day split between 2 primary operators and 4 secondary operators. Each run is comprised of 5 sets and each set has 7 instruments; except for the "off-set", which has 8 instruments. This allows for a total of 144 plates to be processed/day. In between runs, operators prepare reagents for the following shift.

ABI Walk-Through Schedule

Walk Through Schedule	Shift 1 - 6 2:30	10 6:30	Shift 2 2 10:30
0 am Walk Through	X		
10 am Walk Through	X	X	
2 pm Walk Through	X	X	X
6 pm Walk Through		X	X
10 pm Walk Through			X

Five walk-throughs are performed each day (every 4 hours), which consist of checking the status of the ABI 3730xls and unloading plates that have already been processed. Group members also update the arriving shift of problems that may have occurred during the day.

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